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(54) Title: USE OF FIBRONECTIN BINDING PROTEINS IN ORAL HYGIENE (57) Abstract A method of preventing the adherence of pathogenic oral microorganisms to extracellular matrix proteins on surfaces in the oral cavity which method comprises administering an isolated D1-D4 polypeptide from a <i>Staphylococcus aureus</i> Fbp or a monoclonal antibody (Mab), or a fragment thereof, that binds to one or more epitopes of a matrix binding protein, to the oral cavity and oral hygiene compositions for such uses.		

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USE OF FIBRONECTIN BINDING PROTEINS IN ORAL HYGIENE

The present invention relates to the use of anti-adherent fibronectin binding proteins and monoclonal antibodies derived therefrom in oral hygiene and to novel oral hygiene compositions comprising such proteins and monoclonal antibodies.

Pathogenic organisms (bacteria and fungi) in the oral cavity, in particular *S mutans*, *Lactobacillus sp*, *A actinomycetem comitans*, *A viscosus*, *F nucleatum*, *P intermedia*, *E corrodens*, *W recta*, *B forsythus*, *P gingivalis* and *Candida albicans* are responsible for a variety of oral health problems. Oral bacteria adhere to various surfaces in the oral cavity, such as teeth, gingiva soft mucosal tissues and dentures, and form plaque on tooth surfaces. The latter can lead to the development of carious lesions, gingivitis, calculus and periodontal disease. Oral fungi can also give rise to infections in the oral cavity, for instance thrush. Previously, it has been suggested that a suitable therapeutic approach is provided by the use of an anti-microbial agent, such as chlorhexidine, cetyl pyridinium chloride or triclosan which have a bacteriocidal or bacteriostatic effect.

More recently, it has been suggested that an alternative approach may be to use an antiadherent agent, to stop pathogenic organisms from adhering to surfaces within the oral cavity. Teeth become coated with an acquired pellicle which comprises an extracellular protein matrix. This includes specific and non-specific binding sites which are recognised by bacteria and fungi, in particular by receptors located on the surface of the bacterial and fungal cells. If these binding sites are blocked, then cells will be unable to adhere to the extracellular matrix. Thus, for instance, WO 84/04546 (NRDC) discloses the use of a combination of polymers for preventing the adherence of cariogenic bacteria to tooth surfaces whilst EP 0 182 523-A (Imperial Chemical Industries plc) describes certain novel polymers comprising hydrocarbonyl groups with pendant carboxyl and pendant polyalkylene oxide groups which are said to prevent the adherence of cariogenic bacteria on teeth. WO 93/16680 (SmithKline Beecham plc) describes the use of polyvinyl pyrrolidone as a bacterial anti-adherence agent.

The properties of bacterial biofilms have also been studied in the context of infections associated with in-dwelling devices such as catheters. Soon after coming into contact with blood, inert materials, such as used for intravenous cannulae and prosthetic implants, are almost immediately coated with a layer of extracellular matrix proteins, (Cottanaro *et al* 1981, Transactions of the American Society for Artificial Internal Organs 27: 391-395). In particular, this layer includes a plasma protein, fibronectin. It is believed that *staphylococci* are able to bind to fibronectin through bacterial cell surface receptor proteins known as fibronectin binding protein

(Fbp). Some studies have however suggested that blood proteins do not promote adherence of *staphylococci* to biomaterial (eg. Muller *et al* 1991, Infect.Immun. 59: 3323-3326)

5 Fibronectin binding proteins have been isolated from *Staphylococcus aureus* and the nucleotide sequence subsequently established [Signas, C. *et al.* (1989) Proc. Nat. Acad. Sci 86, 699-703 ; Jonsson, K. *et al.* (1991) Eur. J. Biochem. 202, 1041 - 1048] (FbpA and FbpB respectively). The primary fibronectin binding domain of this protein has been identified as a homologous unit (usually of 38 amino acids) that is repeated three times (D1-D3 region) and partially repeated a fourth time (D4
10 region).

EP-0-163-623, EP-0-294-349, EP-0-397-633 and WO-92/02555 disclose certain fibronectin binding polypeptides from *S. aureus* and their use in therapy.

International application WO-94/18327 (SmithKline Beecham plc) discloses *inter alia* the use of a novel polypeptide comprising essentially the D1-D4 region of
15 the fibronectin binding protein found in *S. aureus* J2385 and monoclonal antibodies (Mab) raised there against, to prevent the adhesion of *staphylococci*, such as *S. aureus*, and coagulase-negative staphylococci, such as *S. epidermidis*, to implanted materials and in-dwelling devices such as surgical implants, prosthetic devices and catheters. The region identified as the D1-D4 domain of fibronectin binding protein
20 is considered to be especially relevant in the binding of fibronectin binding protein to fibronectin (Signas. C *et al.* 1989 *op. cit.*).

This International application published after the priority filing of the present application also suggests that the polypeptides and monoclonal antibodies disclosed therein may be used prior to dental treatment, as prophylaxis, in patients with
25 prosthetic joints, to try and avoid bacteraemia, in the context of preventing *S aureus* infections developing on the prosthetic joints. In addition, the application also suggests that, in the context of wound treatment, the polypeptides and monoclonal antibodies disclosed therein may be formulated as a mouthwash. There is however no suggestion that the polypeptides and monoclonal antibodies disclosed therein may
30 be be used to prevent the adherence of oral pathogens to surfaces in the oral cavity.

It has now been unexpectedly found that the polypeptides disclosed in International application WO-94/18327 (SmithKline Beecham plc), may also prevent the adhesion of certain oral pathogens to surfaces in the oral cavity, notably teeth and, therefore, be of use in oral hygiene.

35 Accordingly, the present invention provides a method of preventing the adherence of oral pathogens to surfaces in the oral cavity, in particular tooth surfaces, which method comprises applying a fibronectin binding protein or polypeptide to a patient in need thereof.

The fibronectin binding protein or polypeptide is thus of use in oral hygiene, in particular in combating the plaque - related development of carious lesions, gingivitis, calculus or periodontal disease and in combating oral cavity infections such as candidiasis. The fibronectin binding protein or polypeptide may also have a professional use at the time of oral surgery eg in guided tissue regeneration procedures in periodontal disease or osseointegrated implants to prevent subsequent bacterial infection. It may also be used for irrigation of periodontal pockets and/or incorporated into controlled release devices for administration to the periodontal pocket to prevent or treat periodontal disease progression. It may also be used as a denture cleaner to prevent candidiasis.

The mechanism by which oral pathogens adhere to an oral cavity surface can occur in a number of ways including direct attachment to a surface or by indirect attachment to a surface which may be already coated with a pellicle and/or microorganisms already present on such surface. The fibronectin binding protein or polypeptide can thus prevent the binding of non-pathogenic early colonisers and binding and co-aggregation of an oral pathogen.

The term "preventing the adherence of oral pathogens to surfaces in the oral cavity" therefore means preventing the direct adherence of oral pathogens or preventing the adherence of non-pathogenic early colonisers thereby preventing the subsequent binding and co-aggregation of oral pathogens or even preventing the adherence of pathogenic oral organisms to non-pathogenic early colonisers.

Preferably, the fibronectin binding protein or polypeptide is an isolated D1-D4 polypeptide.

Such a method is preferably carried out in a prophylactic manner, as part of a normal oral hygiene regime.

The term 'oral pathogen' as used herein refers to bacteria and fungi which have been implicated in causing various disease states in the oral cavity, such as caries, gingivitis, periodontitis, calculus and thrush, for instance, *S mutans*, *Lactobacillus sp*, *A actinomycetem comitans*, *A viscosus*, *F nucleatum*, *P intermedia*, *E corrodens*, *W recta*, *B forysthus*, *P gingivalis* and *Candida albicans*. Examples of non-pathogenic early colonisers include *S.sanguis* and *S.gordonii*.

The term 'isolated D1-D4 polypeptide' as used herein refers to a polypeptide consisting of the entire D1, D2, D3 and D4 regions, optionally terminating in PIVP, and optionally having from one to five wall regions (WR) of *S aureus* Fbp, in sequence. Depending upon the host expression system, the polypeptide may include an N-terminal methionine residue.

The above regions correspond to the following regions of FbpA as described in Signas *et al.* (1989), *op. cit.*:

D1	G709-H746	WR1	P843-T856
D2	G747-H784	WR2	P857-T870
D3	G785-S823	WR3	P871-T884
D4	G824-P838	WR4	P885-K898
		WR5	P899-K912

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In a preferred aspect, the polypeptide contains up to three wall regions. Preferred embodiments consist of residues corresponding to residues G709 to T886 and G709 to P838 (optionally where P838→T) of *S aureus* FbpA. The Fbp is preferably from *S aureus* J2385 with the sequence given in Table 2.

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Polypeptides comprising essentially the D1-D4 region of the fibronectin binding protein of *S aureus* J2385 are described in International application no. WO-94/18327 (SmithKline Beecham plc), the contents of which are incorporated herein by reference. *S aureus* J2385 has been deposited at the National Collection of Industrial and Marine Bacteria Ltd. (NCIMB), Aberdeen, Scotland under number

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NCIMB 40532 on 18 th December 1992.

In particular derivatives which are slightly longer or slightly shorter than the peptide of the present invention may be used. In addition, peptides in which one or more of the amino acid residues are modified before or after the peptide is synthesised may be used. Such peptides may, for example, be prepared by

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substitution, addition, or rearrangement of amino acids or by chemical modification thereof. All such substitutions and modifications are generally well known to those skilled in the art of peptide chemistry. A preferred derivative is that whose sequence is shown in SEQ ID No. 6.

The D1-D4 polypeptide may be obtained by expression in *E coli* of the

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plasmid pBROC520. The preparation of this plasmid and the expression and purification of D1-D4 polypeptides are described below in the Examples. The DNA encoding this polypeptide is shown below. Other D1-D4 polypeptides e.g. of FbpA and FbpB, can be similarly expressed by analogous preparation of appropriate plasmids from chromosomal DNA. The DNA encoding the D1-D4 polypeptide of

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FbpA is shown in Table 1 below.

Suitable monoclonal antibodies raised against the proteins and polypeptides hereinbefore described may also be used in oral hygiene therapy.

Accordingly, in a further aspect, the present invention provides a method of preventing the adherence of oral pathogens to surfaces in the oral cavity, in

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particular tooth surfaces, which method comprises applying a monoclonal antibody, or a fragment thereof, that binds to one or more epitopes of a matrix binding protein, such as fibronectin binding protein, to block the adhesion of oral pathogens to the matrix protein to a patient in need thereof.

Such a method is preferably carried out in a prophylactic manner, as part of a normal oral hygiene regime.

The effect of the Mab or fragment is to block the site on the matrix binding protein that is associated with binding to the matrix protein.

5 The invention is particularly concerned with the use of monoclonal antibodies that will prevent the adhesion of oral pathogens, as hereinbefore defined, to surfaces in the oral cavity, in particular to teeth. Accordingly, the monoclonal antibody is preferably directed against epitopes of matrix binding proteins derived from such organisms. Preferably the matrix binding protein to be recognised by the
10 monoclonal antibody is a component of the acquired pellicle.

The preparation of suitable monoclonal antibodies is described in International application no. WO-94/18327 (SmithKline Beecham plc). The antibody may be either intact antibody of M_r approx 150,000 or a derivative of it, for example a Fab fragment or a Fv fragment as described in Skerra, A and
15 Pluckthun, A (1988) Science **240** 1038-1040. If two antigen binding domains are present, each domain may be directed against a different epitope - termed 'bispecific' antibodies.

The monoclonal antibody may be generated initially using as immunogen fibronectin binding protein, or the D1-D4 region of fibronectin binding protein.
20 Fibronectin binding protein of *S aureus* is known to exist in at least two variants FbpA and FbpB [Jonsson *et al.*(1991), *op. cit.*]. The binding domain of either of the above fibronectin binding proteins may be used as immunogen to generate a Mab of this invention.

The antibody or derivative thereof may be prepared by conventional means
25 for example by established monoclonal antibody technology (Kohler, G. and Milstein, C. (1975) , Nature, **256**, 495-497) or using recombinant means e.g. combinatorial libraries, for example as described in Huse, W.D. *et al.*, (1989) Science **246**,1275- 1281.

Preferably the antibody or derivative is prepared by expression of a DNA
30 polymer encoding said antibody in an appropriate expression system. The choice of vector for the expression system will be determined in part by the host, which may be a prokaryotic cell, such as *E. coli* or *Streptomyces sp.* or a eukaryotic cell, such as a mouse C127, mouse myeloma, human HeLa, Chinese hamster ovary, filamentous or unicellular fungi or insect cell. The host may also be a transgenic
35 animal or a transgenic plant [for example as described in Hiatt, A *et al.*, (1989) Nature **34**, 76-78]. Suitable vectors include plasmids, bacteriophages, cosmids and recombinant viruses, derived from, for example, baculoviruses and vaccinia.

The Fab fragment may also be prepared from its parent monoclonal antibody

by enzyme treatment, for example using papain to cleave the Fab portion from the Fc portion.

Phage display technology may also be utilised to select antibody genes with binding activities towards Fbp or D1-D4 either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti-fbp or from naive libraries (McCafferty, J. *et al.*, (1990), *Nature* 348, 552-554; Marks, J. *et al.*, (1992) *Biotechnology* 10, 779-783). The affinity of these antibodies can also be improved by chain shuffling (Clackson, T. *et al.*, (1991) *Nature* 352, 624-628).

Preferably the antibody or derivative thereof is modified to make it less immunogenic in the patient. For example, if the patient is human the antibody may most preferably be 'humanised' ; where the complementarity determining region(s) of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody , for example as described in Jones, P. *et al* (1986), *Nature* 321, 522-525 or Tempest *et al.*, (1991) *Biotechnology* 9, 266-273.

The modification need not be restricted to one of 'humanisation' ; other primate sequences (for example Newman, R. *et al* .1992, *Biotechnology*, 10, 1455-1460) may also be used.

The antibody should be screened again for high affinity to Fbp, D1-D4 polypeptide and/or fusion protein.

As mentioned above, a fragment of the final antibody may be prepared.

A method involving the use of the humanised monoclonal antibody, or its fragment having binding activity, forms a further aspect of this invention.

A DNA "coding sequence of" or a "nucleotide sequence encoding" a particular protein, is a DNA sequence which is transcribed and translated into a polypeptide when placed under the control of appropriate regulatory sequences.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bound at the 3' terminus by a translation start codon (e.g., ATG) of a coding sequence and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

DNA "control sequences" refers collectively to promoter sequences,

ribosome binding sites, polyadenylation signals, transcription termination sequences, upstream regulatory domains, enhancers, and the like, which collectively provide for the expression (i.e., the transcription and translation) of a coding sequence in a host cell.

- 5 A control sequence "directs the expression" of a coding sequence in a cell when RNA polymerase will bind the promoter sequence and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

- 10 A "host cell" is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous DNA sequence.

- A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes and yeasts, for example, the exogenous DNA may be
15 maintained on an episomal element, such as a plasmid. With respect to eukaryotic cells, a stably transformed or transfected cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of
20 daughter cell containing the exogenous DNA.

 A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

- 25 A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature.

- This invention provides an isolated nucleic acid molecule encoding the polypeptide. The isolated nucleic acids particularly the DNAs can be introduced into expression vectors by operatively linking the DNA to the necessary expression
30 control regions (e.g. regulatory regions) required for gene expression. The vectors can be introduced into the appropriate host cells such as prokaryotic (e.g., bacterial), or eukaryotic (e.g. yeast, insect or mammalian) cells by methods well known in the art (Ausubel et al., *supra*). The coding sequences for the desired proteins having been prepared or isolated, can be cloned into a suitable vector or
35 replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Examples of recombinant DNA vectors for cloning and host cells which they can transform include the bacteriophage λ (*E. coli*), pBR322 (*E. coli*), pACYC177 (*E. coli*),

pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-*E. coli* gram-negative bacteria), pHV14 (*E. coli* and *Bacillus subtilis*), pBD9 (*Bacillus*), pIJ61 (*Streptomyces*), pUC6 (*Streptomyces*), YIp5 (*Saccharomyces*), a baculovirus insect cell system, , YCp19 (*Saccharomyces*). See, generally, "DNA Cloning": Vols. I & II, Glover *et al.* ed. IRL Press Oxford (1985) (1987) and; T. Maniatis *et al.* ("Molecular Cloning" Cold Spring Harbor Laboratory (1982).

The gene can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control" elements), so that the DNA sequence encoding the desired protein is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. The polypeptides of the present invention can be expressed using, for example, the *E. coli* *tac* promoter or the protein A gene (*spa*) promoter and signal sequence. Leader sequences can be removed by the bacterial host in post-translational processing. See, e.g., U.S. Patent Nos. 4,431,739; 4,425,437; 4,338,397.

In addition to control sequences, it may be desirable to add regulatory sequences which allow for regulation of the expression of the protein sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

An expression vector is constructed so that the particular coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the control sequences being such that the coding sequence is transcribed under the "control" of the control sequences (i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence). Modification of the coding sequences may be desirable to achieve this end. For example, in some cases it may be necessary to modify the sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

In some cases, it may be desirable to add sequences which cause the

secretion of the polypeptide from the host organism, with subsequent cleavage of the secretory signal.

A number of prokaryotic expression vectors are known in the art. See, e.g., U.S. Patent Nos. 4,578,355; 4,440,859; 4,436,815; 4,431,740; 4,431,739; 4,428,941; 4,425,437; 4,418,149; 4,411,994; 4,366,246; 4,342,832; see also U.K. Patent Applications GB 2,121,054; GB 2,008,123; GB 2,007,675; and European Patent Application 103,395. Yeast expression vectors are also known in the art. See, e.g., U.S. Patent Nos. 4,446,235; 4,443,539; 4,430,428; see also European Patent Applications 103,409; 100,561; 96,491. pSV2neo (as described in J. Mol. Appl. Genet. 1:327-341) which uses the SV40 late promoter to drive expression in mammalian cells or pCDNA1neo, a vector derived from pCDNA1 (Mol. Cell Biol. 7:4125-29) which uses the CMV promoter to drive expression. Both these latter two vectors can be employed for transient or stable (using G418 resistance) expression in mammalian cells. Insect cell expression systems, e.g., *Drosophila*, are also useful, see for example, PCT applications WO 90/06358 and WO 92/06212 as well as EP application EP0290261.

Depending on the expression system and host selected, the polypeptide of the present invention may be produced by growing host cells transformed by an expression vector described above under conditions whereby the polypeptide of interest is expressed. The polypeptide is then isolated from the host cells and purified. If the expression system secretes the polypeptide into growth media, the polypeptide can be purified directly from the media. If the polypeptide is not secreted, it is isolated from cell lysates or recovered from the cell membrane fraction. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

In a further aspect, the present invention provides an oral hygiene composition comprising an above mentioned fibronectin binding protein or polypeptide, in particular an isolated D1-D4 polypeptide or Mab or active fragment and an orally acceptable carrier.

Suitable oral hygiene compositions are well known in the art and include dentifrices, including opaque and transparent/translucent toothpastes, liquid dentifrices, non-abrasive gels, mouthwashes, mouthrinses and gargles, mouthsprays, gingival irrigating devices, paint-on varnishes, formulations to be sucked or chewed by the user such as gums and lozenges and dental flosses.

Such compositions will contain appropriate formulating agents such as abrasives, surfactants, humectants, thickening agents, flavouring agents, sweetening agents, opacifying agents, preservatives and water, selected from those conventionally used in the oral hygiene composition art for such purposes and which

are compatible with the polypeptide or monoclonal antibodies hereinbefore described.

Suitable surfactants for use in compositions according to the present invention include, for instance, anionic, nonionic, cationic and amphoteric
5 surfactants or mixtures thereof.

Suitable anionic surfactants include alkali metal (C₁₂₋₁₈)alkyl sulphates, for instance sodium lauryl sulphate, and N-acyl sarcosinates and N-acyl taurines in which the acyl moiety has from 12 to 16 carbon atoms, for instance, N-lauroyl, N-myristoyl and N-palmitoyl sarcosine alkali metal salts.

10 Suitable nonionic surfactants include, for example, polyethoxylated sorbitol esters, in particular polyethoxylated sorbitol monoesters, for instance, PEG(40) sorbitan di-isostearate, and the products marketed under the trade name 'Tween' by ICI; polycondensates of ethylene oxide and propylene oxide (poloxamers), for instance the products marketed under the trade name 'Pluronic' by
15 BASF-Wyandotte; condensates of propylene glycol; polyethoxylated hydrogenated castor oil, for instance, cremophors; and sorbitan fatty esters.

Suitable amphoteric surfactants include, for example, long chain imidazoline derivatives such as the product marketed under the trade name 'Miranol C2M' by Miranol; long chain alkyl betaines, such as the product marketed under the
20 tradename 'Empigen BB' by Albright + Wilson, and long chain alkyl amidoalkyl betaines, such as cocamidopropylbetaine, and mixtures thereof.

Suitable cationic surfactants include the D,L-2-pyrrolidone-5-carboxylic acid salt of ethyl-N-cocoyl-L-arginate, marketed under the trade name CAE by Ajinomoto Co. Inc., and cocamidopropyl PG dimonium chloride phosphate and
25 lauramidopropyl PG dimonium chloride phosphate, available under the trade names Monaquat PTC and Monaquat PTL, respectively, from Mona Corporation.

Advantageously, the surfactant is present in the range 0.005 to 20%, preferably 0.1 to 10%, more preferably 0.1 to 5% by weight of the dentifrice.

Suitable thickening agents include, for instance, nonionic thickening agents
30 such as, for example, (C₁₋₆)alkylcellulose ethers, for instance methylcellulose; hydroxy(C₁₋₆)alkylcellulose ethers, for instance hydroxyethylcellulose and hydroxypropylcellulose; (C₂₋₆)alkylene oxide modified (C₁₋₆)alkylcellulose ethers, for instance hydroxypropyl methylcellulose; and mixtures thereof. Other thickening agents such as natural and synthetic gums or gum like material such as Irish Moss,
35 gum tragacanth, sodium carboxymethylcellulose, polyvinyl pyrrolidone, starch, xanthan, carrageenan and thickening silicas may also be used.

Advantageously the thickening agent is present in the range 0.01 to 30%, preferably 0.1 to 15%, more preferably 1 to 5%, by weight of the composition.

Suitable humectants for use in compositions of the invention include for instance, glycerine, sorbitol, propylene glycol or polyethylene glycol, or mixtures thereof; which humectant may be present in the range from 5 to 90%, preferably 5 to 70%, more preferably 10 to 50% by weight of the dentifrice.

5 Suitable abrasives for use in dentifrice compositions of the present invention include calcium carbonate, calcium phosphates, calcium pyrophosphate, insoluble sodium metaphosphate, sodium aluminosilicate, alumina, hydrated alumina, zinc orthophosphate, plastic particles, and silica, of which silica is the preferred abrasive.

10 Suitable silicas include natural amorphous silicas, such as, for instance, diatomaceous earth, and synthetic amorphous silicas, such as precipitated silicas and silica gels, including silica xerogels. Suitable silica xerogels are described in US 3,538,230. Suitable grades of precipitated silicas have BET surface areas in the range 20 to 300, preferably 20 to 100 m²/g and median agglomerate sizes in the
15 range 2 to 50, preferably 5 to 30µ.

Suitable precipitated silicas and silica xerogels are those marketed under the trade names Sident and Syloblanc, by Degussa and W R Grace Corporation Davison Chemical Division, respectively.

20 Advantageously, the silica is a "low anion" silica. As used herein, the term "low-anion" silicas refers to those in which anionic impurities such as sodium sulphate and sodium silicate which normally arise during the course of the manufacturing process are kept to a minimum, through careful control of the manufacturing process. "Low anion" silicas suitably have less than 1%, preferably less than 0.5% advantageously less than 0.25% by weight of anionic impurities.

25 Suitable such "low anion" silicas are described in EP 0 368 130 (Proctor & Gamble), EP 0 315 503 and EP 0 396 459 (Rhone-Poulenc) and WO 90/05113 (J.M. Huber Corp). Alternatively, grades of commercially available silica with ionic impurities may be rendered suitable by washing thereof with deionised water. Conductivity measurements on the water after washing may be used to monitor the
30 efficacy of such washing. Suitably the conductivity of the water after washing is reduced to less than 200µSiemens/cm. Suitable "low anion" silicas include the grade RP93 available from Rhone-Poulenc.

Suitably, compositions will have from 5 to 80%, preferably from 10 to 60% by weight of the abrasive.

35 Suitable mouthwash formulations will have an aqueous base comprising water or aqueous ethanol, and optionally a further liquid such as glycerin or propylene glycol. A surfactant may also be included, to improve the sensory properties of the composition. Mouthwash compositions may be provided in a

"ready to use" form; as a concentrated solution, for dilution by the user immediately prior to use; or in solid form, such as a tablet or in a sachet, for dissolution by the user immediately prior to use. Tablets may suitably be prepared using xylitol and/or sorbitol as the major ingredient. The sachets and tablets may
5 be formulated to provide, on dissolution, a still mouthwash, or, by the incorporation of a suitable effervescent couple, for instance sodium carbonate/bicarbonate and citric acid, an effervescent mouthwash.

Oral hygiene compositions of the present invention may usefully further comprise an anti-caries agent, for instance a source of fluoride ions such as an alkali
10 metal or amine fluoride salt, for example sodium fluoride, tin (II) fluoride. Alternatively, the fluoride ion source may be an alkali metal monofluorophosphate salt, for example sodium monofluorophosphate, optionally used in combination with an agent such as calcium glycerophosphate which is known to enhance the activity of monofluorophosphate (GB 1 384 375, Beecham Group). Suitably the
15 composition will comprise between 100 and 2500ppm, preferably 200 and 1500ppm of fluoride ions.

Oral hygiene compositions of the present invention may also comprise other active agents conventionally used in oral hygiene compositions, for instance:
an anti-plaque agent such as chlorhexidine, cetyl pyridinium chloride, triclosan,
20 histatin or nisin (particularly in the purified form available as Ambicin N from Applied Microbiology Inc., New York);
an anti-calculus agent such as a tetra- or a di-alkali metal pyrophosphate salt, or a mixture thereof, an alkali metal tripolyphosphate salt or an azacycloheptane diphosphonate salt; or
25 an anti-sensitivity agent such as strontium acetate, strontium chloride or a potassium salt such as potassium nitrate, potassium chloride or potassium citrate.
Such agents will be included at levels to provide the desired therapeutic effect.

It will be appreciated that the mouthwashes disclosed generically in International application no. WO-94/18327 (SmithKline Beecham plc) comprise the
30 above isolated D1-D4 polypeptide or Mab or active fragment. There is however no suggestion to incorporate a further oral hygiene agent. Accordingly, in a further aspect, the present invention provides novel mouthwashes which comprise the above isolated D1-D4 polypeptide or Mab or active fragment in combination with a further oral hygiene agent, such as an anticaries, antiplaque, anticalculus and/or
35 antisensitivity agent, as hereinbefore described and an orally acceptable carrier. Furthermore, there is no disclosure of specific mouthwash formulations. Accordingly, in a further aspect, the present invention provides novel mouthwashes which comprise the above isolated D1-D4 polypeptide or Mab or active fragment in

an orally acceptable carrier which is essentially free from alcohol.

Oral hygiene compositions according to the present invention will have a pH which is orally acceptable, for instance in the range pH 5 to 10.

Oral hygiene compositions according to the present invention may be prepared by admixing the ingredients in the appropriate relative amounts in any order that is convenient and thereafter and if necessary adjusting the pH to give the final desired value.

It is expected that the daily 'dosage' level of the polypeptide or monoclonal antibody for normal usage will be from 0.01 to 10 mg/kg, typically around 1 mg/kg. The polypeptide or monoclonal antibody will be incorporated into oral hygiene compositions according to the present inventions at concentrations sufficient to provide the aforementioned daily rate eg 0.002% to 2.3%, preferably 0.23% (w/w).

With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their use.

The efficacy of a fibronectin binding protein, D1-D4, in preventing the adherence of organisms found in the oral cavity was demonstrated using the following assays. D1-D4 (709-886) referred to in the Examples relates specifically to the compound shown in SEQ 1D No. 6.

Streptococcus/hydroxyapatite adherence radioisotope assay

A. Labelling and preparation of culture

Tritiated thymidine (30 μ Ci) (Amersham TRA.310) was added to Todd Hewitt broth (18ml). This was inoculated with *Streptococcus sanguis* NCTC 10904 (or *Streptococcus mutans* NCTC 11061) and incubated overnight at 30°C. The culture was centrifuged and washed three times in phosphate buffered saline (PBS) and then resuspended in PBS (approximately 10⁹ cells/ml).

B. Preparation of hydroxyapatite (HA)

Hydroxyapatite (20mg) was weighed out into bijoux bottles and incubated for 6 hours in PBS at room temperature and then washed twice in PBS. The HA was then incubated in 2ml of pooled clarified saliva (or fibronectin) overnight at room temperature and then washed three times in PBS. The HA was transferred to a scintillation vial and PBS (2ml) containing D1D4 (709-886) (100 μ g/ml) was added and then incubated for 1 hour at 37°C. The HA was finally washed three times in PBS. A control was also set up whereby only PBS was added to the saliva or fibronectin coated HA in place of D1D4.

C. Performance of adherence assay

- The resuspended radiolabelled culture (approximately 10^9 cells/ml in PBS) (2ml) was added to the (optionally D1D4 treated) HA in the scintillation vial and incubated at 37°C for 3 hours. The HA was then washed three times in PBS before being transferred to a new scintillation vial and washed twice in PBS. Optiphase Hisafe II scintillation fluid (10ml) was added and counts per minute recorded in a Wallac 1209 RACKBETA scintillation counter.

- 10 The following results were obtained:

HA coating	Organism	% reduction in adherence
		Compared to control with no D1D4
saliva	<i>Streptococcus sanguis</i>	27
saliva	<i>Streptococcus mutans</i>	71
fibronectin	<i>Streptococcus sanguis</i>	25
fibronectin	<i>Streptococcus mutans</i>	90

These results indicate that D1D4 (709-886) significantly reduces the adherence of *S.sanguis* or *S.mutans* to HA coated with saliva or fibronectin.

15

Biotinylated Bacterial MT Assay

A. Growth of streptococcal cells

- Three 100ml volumes of Brian Heart Infusion broth were inoculated singularly with either *S sanguis* (NCTC 10904), *S mutans* (NCTC 11061) or *S gordonii* (NCTC 7865). These were grown overnight at 37°C and then centrifuged and washed 3 times in PBS. After the final washing the suspension was again centrifuged and the supernatant discarded and the pellet stored at 4°C and transferred to -40°C a few hours later.

25

B. Preparation of biotinylated streptococcal cells

a. *S. mutans* NCTC 11061

- Approx. 10^{10} cells prepared as described above were resuspended in 16 ml PBS 'A' (Dulbecco). 10 ml of this solution was mixed thoroughly with 0.5 ml NHS-biotin (Pierce; 20 mg/ml in DMSO) and left for approx 1.5 hours at room temperature.

30

temperature. The cells were then washed twice with PBS, using centrifugation at 4000 rpm to pellet the cells each time, and were finally resuspended in 10 ml PBS, aliquoted and stored frozen at -40 degrees C.

5 **b. *S. gordonii* NCTC 7865**

The synthesis of biotinylated *S. gordonii* followed similar methodology to that described for *S. mutans*.

c. *S. sanguis* NCTC 10904

- 10 The synthesis of biotinylated *S. sanguis* followed similar methodology to that described for *S. mutans*.

C. Adhesion assay using streptococcal cells

- All steps except washing steps were carried out static at 37°C. Generally one
15 aliquot of the frozen cells described above was thawed for a single experiment, and resuspended as described below.

- Flat bottomed MT plates (Nunc Microwell 96F) were coated with 250 microlitres of
 a 1.0mg/ml gelatin solution per well (United States Biochemical Co.) for 1h.
20 Plates were then aspirated and coated with 250 microlitres well of 10µg/ml human
 fibronectin in PBS for 1h and washed four times using 0.02M NaH₂PO₄/0.3M
 NaCl/0.5%(v/v) Tween 80 pH7.4 (Wash buffer). Standards or test samples of D1-
 D4 at various dilutions in PBS (100microlitres/well) were added for 1h. Plates
 were then washed four times in Wash buffer and 100 microlitres/well 1x10⁷cells/ml
25 biotinylated streptococci in PBS were added for 1h. Plates were washed again four
 times with Wash buffer and 100microlitres/well 1:2000 Streptavidin alkaline
 phosphatase (Amersham) in wash buffer containing 0.5% bovine serum albumin
 (Fraction V, Sigma) was added for 30min. Plates were then washed four times
 with Wash buffer and 100microlitres/well of 1.0mg/ml p-nitrophenol phosphate
30 (Sigma) was added until the yellow colour developed to an acceptable level ,
 generally about 30min. The optical density of the solutions was then read at
 405nm.

- In a similar manner MT cells coated with gelatin/saliva or with saliva were
35 prepared. Saliva was isolated from 3 donors, pooled, filtered (0.22 µm) and used
 undiluted within 2 hours.

 The following results using D1-D4 (709-886) were obtained.

- 40 OD at 405 nm (n=3, figures in parenthesis refer to SD)

<i>S.gordonii</i>	D1-D4 (ug/ml)			
	0	10	100	1000
Gelatin /Fibronectin	0.98 (0.04)	0.68 (0.05)	0.51 (0.06)	0.43 (0.04)
Gelatin/Saliva	0.49 (0.01)	0.43 (0.01)	0.38 (0.03)	0.33 (0.02)
Saliva	0.37 (0.03)	0.32 (0.08)	0.26 (0.01)	0.24 (0.03)

<i>S.mutans</i>	D1-D4 (ug/ml)			
	0	10	100	1000
Gelatin /Fibronectin	1.00 (0.09)	0.80 (0.09)	0.63 (0.07)	0.78 (0.17)
Gelatin/Saliva	0.39 (0.02)	0.40 (0.01)	0.38 (0.01)	0.36 (0.01)
Saliva	0.32 (0.01)	0.31 (0.05)	0.29 (0.02)	0.26 (0.02)

5

<i>S.sanguis</i>	D1-D4 (ug/ml)			
	0	10	100	1000
Gelatin /Fibronectin	0.71 (0.15)	0.54 (0.08)	0.48 (0.02)	0.48 (0.01)
Gelatin/Saliva	0.47 (0.01)	0.46 (0.02)	0.62 (0.08)	0.59 (0.05)
Saliva	0.61 (0.02)	0.48 (0.06)	0.54 (0.02)	0.46 (0.04)

These results indicated that D1-D4 (709-886) reduces the adherence of *S.gordonii*, *S.mutans*, *S.sanguis* to fibronectin or saliva.

10

The following Preparations and Examples illustrate the preparation of D1-D4 polypeptides for use as antigens and as anti-adhesive agents.

Preparation of key reagents

15

- a) Construction of vector pBROC413 - The plasmid pT7-7 [Tabor, S (1990), Current Protocols in Molecular Biology, F.A.Ausubel, Brent,R.E. Kingston,D.D. Moore,J.G. Seidman,J.A. Smith, and K.Struhl,eds.] pp.16.2.1-

16.2.11. Greene Publishing and Wiley-Interscience, New York.] contains DNA corresponding to nucleotides 2065-4362 of pBR322 and like pBR322 can be mobilized by a conjugative plasmid in the presence of a third plasmid ColK. A mobility protein encoded by ColK acts on the *nic* site at nucleotide 2254 of pBR322 initiating mobilization from this point. pT7-7 was digested with *LspI* and *BglII* and the protruding 5' ends filled in with the Klenow fragment of DNA Polymerase I. The plasmid DNA fragment was purified by agarose gel electrophoresis, the blunt ends ligated together and transformed into *E. coli* DH1 by electroporation using a Bio-Rad Gene Pulser and following the manufacturers recommended conditions. The resultant plasmid pBROC413 (Fig. 1) was identified by restriction enzyme analysis of plasmid DNA.

The deletion in pBROC413 from the *LspI* site immediately upstream of the ϕ 10 promoter to the *BglII* site at nucleotide 434 of pT7-7 deletes the DNA corresponding to nucleotides 2065-2297 of pBR322. The *nic* site and adjacent sequences are therefore deleted making pBROC413 non mobilizable.

b) Preparation of biotinylated fibronectin probe - Fibronectin (FN) was purified from a human plasma fraction (a gift from Dr. D. Pepper, Scottish Blood Transfusion Service) on Gelatin Sepharose, essentially as described by Miekka *et al* (1982) Thromb. Res. 27, 1-14.

11.2mg FN (2.0ml) in 0.05M Tris/0.1M NaCl pH7.5 was made 1.0mg/ml by addition of 9.2ml 0.05M Na₂B₄O₇ pH8.6 buffer. 80 μ l of N-hydroxysuccinimidobiotin (Amersham UK) from a 5.0mg/ml stock in dry dimethylformamide was added and the mixture incubated at room temperature for 1h with constant agitation. The reaction was terminated by buffer exchange into Dulbecco's 'A' phosphate-buffered saline containing 0.1% (w/v) bovine serum albumin (BSA) using five Sephadex G25 columns (PD10, Pharmacia).

c) Synthesis of Fibronectin Sepharose CL4B - 100 mg of human fibronectin, purified as described above, was coupled to 7 g of Cyanogen bromide-activated Sepharose CL4B (Pharmacia) at room temperature according to the manufacturers instructions, to yield 25 ml of gel matrix. Before use the FN-Sepharose was washed with all buffers used in the subsequent purification.

Numbering of amino acid residues in the Examples

In the following examples the numbering of amino acid residues corresponds to the residues of FbpA according to Signas *et al.*, (1989) *op. cit.* Residues 709-838 of FbpA correspond to residues 1-130 of the sequence of *S. aureus* J2385 given

in Table 2 and residues 709-886 correspond to residues 1-174.

Preparation 1 - Isolation of DNA from *Staphylococcus aureus* J2385 coding for the fibronectin binding domains of Fibronectin Binding Protein.

- 5 *S. aureus* J2385 is strain B described in Cookson *et al.* [1987] THE LANCET of August 15th. page 387. It is a clinical strain derived from a skin lesion. Chromosomal DNA was prepared by treating cells collected from an overnight shake flask culture with lysostaphin to lyse them and phenol/chloroform to remove cell protein. From this unpurified DNA preparation the fragment of DNA encoding
- 10 the fibronectin binding domains of Fibronectin Binding Protein was obtained by a PCR amplification reaction. The oligonucleotide primers used in the PCR reaction were:-

FIB 1 5'-GGGAATTCATATGGGCCAAAATAGCGGTAACCAGTC-3'
 FIB 2 5'-GCGGATCCTTACGTTGGTGGCACGATTGGAGGTG-3'

- 15 PCR amplification was carried out using *S. aureus* J2385 chromosomal DNA (10ng.) FIB 1 (1 micromolar), FIB 2 (1 micromolar), Tris-HCl pH8.3 (10 mM), KCl (50mM), MgCl₂ (1.5 mM), gelatin (0.001 %), Na dGTP (200 micromolar) Na dATP (200 micromolar), Na dTTP (200 micromolar), Na dCTP (200 micromolar) and Taq DNA Polymerase (2.5 units) in a final volume of 100
- 20 microlitres made up with distilled water. The aqueous solution was overlaid with 80 microlitres of liquid paraffin and subjected to 30 cycles of 94 °C (1min.), 60°C (1 min.) and 72°C (2 min.) to enable the amplification to occur. When 10 microlitres of the aqueous reaction were examined after amplification on 1.5 % agarose gel electrophoresis in the presence of ethidium bromide (0.5
- 25 micrograms/ml.) it was observed by comparison to a sample of DNA fragments of known size that a single species of DNA fragment of approximately 500 basepairs was obtained.

- Preparation 2 - Obtaining the sequence of the DNA fragment obtained by PCR amplification of *S. aureus* J2385 chromosomal DNA using primers FIB 1 and FIB 2.**
- 30

- The size of the PCR fragment obtained (approx. 500 bp.) using conditions described in Preparation 1 was unanticipated as the primers FIB 1 and FIB 2 were designed to be homologous to sites in Fibronectin Binding Protein gene (as described by Signas
- 35 C., *et al.* [1989] P.N.A.S. USA vol 86,699-703) of *S. aureus* which were reported to exist approximately 400 bp. apart. In order to authenticate the nature of the

DNA fragment it was cloned into pUC19 and sequenced. The DNA from one PCR reaction was incubated with Eco RI and Bam HI restriction enzymes and cloned into similarly treated pUC19 [Yanisch -Perron, C. *et al.* (1985) *Gene*, 33, 103] and sequenced to create pBROC 519a. Because the PCR process occasionally
5 introduces deletions and base substitutions into amplified DNA, similar clones of J2385 DNA were retained to cosequence along with pBROC 519a for confirmation of results. pBROC 519a was sequenced in both strands using the SEQUENASE II kit obtained from United States Biochemical. The sequence obtained revealed the cloned fragment encoded 524bp of *S. aureus* J2385 DNA. When the sequence was
10 compared with that published by Signas *et al.* (*op. cit.*) it was apparent that it was extensively homologous to region 2350-2885 of that *S. aureus* gene (using the Signas *et al.* numbering) but possessed significant differences (see Table 1). In particular, the derived amino acid sequence displayed amino acid differences to the published sequence at residues 752 (ASN→ASP), 803 (SER→ASN), 821
15 (LYS→GLN), 825 (GLN→HIS) and a four amino acid deletion (838→841), see Table 2. These differences were confirmed by sequencing independent clones of PCR amplified DNA. When the derived amino acid sequence from *S. aureus* J2385 DNA was compared to the corresponding regions in *S. aureus* Fibronectin binding protein type A (Signas *et al.* [1989]) and *S. aureus* type B protein (Jonsson *et al.*
20 [1991] *EUR. J. BIOCHEM.* vol 202 pages 1041-1048) it was clear that the fibronectin binding domains of the *S. aureus* J2385 protein was very similar to the respective domains of both the type A and type B proteins but was identical to neither (Table 2). This suggested that the *S. aureus* J2385 protein should be denoted type C.

25

Preparation 3 - Preparation of pBROC 520 in *E. coli* BL21 (DE3)

The T7 polymerase/promoter expression system (as described by Tabor S. in Current Protocols in Molecular Biology, F.A.Ausubel, R.Brent, R.E.Kingston, D.D.Moore, J.G.Seidman, J.A.Smith and K.Struhl, eds., pp.16.2.1-16.2.11.
30 Greene Publishing and Wiley-Interscience, New York) was used to express the protein fragment encoded by the cloned *S. aureus* J2385 derived DNA. The host was *E.coli* BL21(DE3) which inducibly expresses the T7 polymerase gene (see Studier F.W. and Moffat B.A. [1986] *J.Mol.Biol.* vol.189,113-130).

The 0.5 kb BamHI/NdeI fragment of *S.aureus* J2385 derived DNA was
35 isolated from pBROC 519a (3 microgrammes) by restriction enzyme digestion and electrophoresis on low melting point agarose gel. This material (100 nanogrammes) was used in a ligation reaction with BamHI/NdeI digested pBROC 413 DNA (500 nanogrammes). The ligated DNA was electrotransformed into *E.coli* Delta M15 (see

Sambrook, J., Fritsch, E.F. and Maniatis, T. editors [1989] Molecular Cloning, A Laboratory Manual (second edition) page 2.57 for details of the lacZ Delta M15 mutation) and transformants selected on LB agar containing ampicillin (50 microgrammes/ml.).

- 5 Plasmid DNA preparations were made from five ampicillin resistant colonies and were all shown by restriction enzyme site mapping to be pBROC 413 carrying the *S.aureus* derived DNA fragment. One of the plasmid preparations (designated pBROC 520) was used to transform *E.coli* BL21(DE3) to give the desired expression construct/host combination. pBROC 520 encodes the peptide given in
10 SEQ ID NO 6, hereinafter referred to as D1-D4(709-886).

Preparation 4 - Construction of pBROC 533, a plasmid expressing a polypeptide predominantly consisting of the D1-D4 domains of *S. aureus* J2385 Fibronectin Binding Protein.

- 15 The following oligomeric polynucleotide (A) and its complement were synthesised on a Pharmacia LKB Gene Assembler Plus DNA synthesiser:-

CGGAATTCGT CAACAAACGA TTGAAGAAGA TACAACGACG
TAAGATCTGG ATCCGCATGC GAATTCCG

20

- Samples of the two oligonucleotide preparations (0.14 microgrammes of each) were mixed, heated to 94 degrees C for 5 minutes and then allowed to anneal at 37 degrees C for 10 minutes. The double stranded DNA was digested with EcoRI enzyme and subsequently cloned into the EcoRI site of pUC19 (2
25 microgrammes of EcoRI digested vector/ligation) to give pBROC 528 in *E. coli* Delta M15.

The BamHI digested kanamycin resistance marker of pUC4K (obtained from Pharmacia, code no. 27-4958-01) was then cloned into the unique BglII site of the resulting plasmid construct pBROC 528 to give pBROC 529.

- 30 pBROC 529 plasmid DNA (5microgrammes) grown in *E. coli* Delta M15 was double digested with HincII/SphI and the approximately 1.4kb DNA fragment encoding the kanamycin resistance gene and that moiety of Fibronectin Binding Protein encoded by the oligonucleotide originally synthesised was isolated using low melting point agarose/agarase. This DNA was used in a ligation reaction with
35 partially digested HincII, completely digested SphI plasmid DNA (1 microgramme) of pBROC 519a (see Example 2). The ligation products were electrotransformed into *E. coli* Delta M15, selecting for kanamycin resistance. In this way, it proved possible to isolate pBROC 530, a plasmid which as ascertained by sequencing across

the HincII sites of the staphylococcal DNA and by restriction mapping, carried a DNA fragment encoding the D1-D2-D3-D4 regions (residues 1-129 in Table 2) of the Fibronectin Binding Protein of *S. aureus* J2385. The DNA fragment additionally coded for a threonine residue at the carboxy terminal of the polypeptide.

Next, the staphylococcal DNA of pBROC 530 was removed from the plasmid vector by NdeI/BamHI digestion of a plasmid DNA preparation and cloned into similarly digested pBROC413 (see Key reagents a) to give pBROC 531.

pBROC 531 was grown in a transformed strain of *E. coli* Delta M15 and then digested with SalI to remove the kanamycin resistance gene and subsequently religated to create pBROC 533. This step was carried out to prevent unnecessary overexpression of the kanamycin resistance gene from the T7 promoter in pBROC 531 as it was considered that this would be detrimental to maximal expression of the staphylococcal DNA.

pBROC 533 was transformed into *E. coli* BL21(DE3) to furnish *E. coli* BL21(DE3), pBROC533.

A variant of plasmid pBROC 531 may alternatively be constructed using the following oligomeric polynucleotide (B) and its complement:-

CGGAATTCGT CAACAAACGA TTGAAGAAGA TACAACGCCG
TAAGATCTGG ATCCGCATGC GAATTCCG

This gives the same polypeptide as above with the terminal threonine replaced by proline (SEQ ID no 8).

Example 1 - Expression, isolation and purification of D1-D4 (709-886)

polypeptide of J2385 expressed from pBROC 520 in *E. coli* BL 21 (DE3)

a) Expression - Single colonies of *E. coli* BL 21 (DE3) harbouring either pBROC 413 (non-coding) plasmid or pBROC 520 (encoding D1-D4 (709-886)) were inoculated into 30 ml capped containers (universals) containing 10 ml of NZCYM medium (1% (w/v) Bactotryptone, 0.5% (w/v) Bacto yeast extract, 0.5% (w/v) NaCl, 0.1% (w/v) casamino acids and 0.2% (w/v) $MgSO_4 \cdot 7 H_2O$ pH 7.0) and 75 µg/ml ampicillin. The cultures were incubated at 37°C, 230 rpm overnight. The overnight cultures were used to inoculate 250 ml NZCYM medium containing 150 µg/ml ampicillin. The cultures were incubated at 37°C, 230 rpm until A_{600} reached 0.5 absorbance units. The cultures were then induced with 1mM IPTG (Isopropylthio-β-D-galactoside) and incubated under the same conditions for a further 4 hours. 1 ml samples were removed pre-induction and 1,2,3 and 4 hours

post-induction. Each sample was spun in an eppendorf centrifuge for 1 minute after which the supernatant was removed. The pellets were then resuspended in 100µl of reducing buffer (50mM Tris. HCl pH6.8, 100mM dithiothreitol (DTT), 0.1 % (w/v) bromophenol blue, 2 % (w/v) SDS, 10 % (v/v) glycerol) or non-reducing buffer (DTT is omitted). The samples were heated for 3 mins at 90°C before being stored at -40°C.

b) **Detection of expressed product - *E. coli* resuspended pellets** containing either pBROC 413 (non-coding plasmid) or pBROC 520 (encoding D1-D4 (709-886)) were separated on sodium dodecyl sulphate containing 4-20 % polyacrylamide gels (Novex, British Biotechnology Ltd.), essentially as described by the manufacturers. Separated proteins were transferred to Immobilon (Millipore (UK) Ltd.) using Sartoblot II blotting apparatus according to the manufacturer's instructions. Unreacted sites on the blot were blocked by incubation in 10mM NaH₂PO₄/0.15M NaCl/0.02 % (w/v) Ficoll/0.02 % (w/v) polyvinylpyrrolidone/0.1 % (w/v) bovine serum albumin (BSA) pH7.4 for 1h at room temperature with constant agitation. The blot was probed with biotinylated fibronectin at 200 µg/ml in 0.02M NaH₂PO₄/0.3M NaCl/0.5 % (w/v) Tween 80/1.0 % (w/v) BSA pH7.4 for 4h at room temperature with constant agitation. Bands were visualised using a Streptavidin Gold/silver stain system (Amersham UK) according to the manufacturer's instructions. Putative D1-D4 (709-886) polypeptide was identified as a new band in the pBROC 520 lane.

c) **Isolation of solubilised D1-D4 (709-886) - Frozen cell pellet of *E. coli* BL21 DE3 (pBROC 520))** (from a 300 ml culture) prepared essentially as described in a) above and using an induction period of 3h was allowed to thaw at 4 °C for 2h and was then resuspended in 50 mM Tris/50 mM NaCl/1 mM EDTA/0.1 mM phenyl methyl sulphonyl fluoride (PMSF) pH 8.0 (30 ml). The suspension was transferred to a 100ml glass beaker and sonicated (Heat Systems - Ultrasonics W380; 70 Watts, 50 x 50 % pulse, pulse time = 5 sec). The sonicate was immediately centrifuged (6000g / 4°C/ 10 min) and the pellet discarded. The supernatant, containing the solubilised D1-D4 (709-886), was adjusted to pH 7.4 and retained at -40°C.

d) **Purification of D1-D4 (709-886) product**

(i) D1-D4 (709-886) supernatant, prepared as described above, was applied to a FN-Sepharose column (1.6 x 13.2 cm) equilibrated in Dulbecco's 'A' phosphate-buffered saline (PBS)/0.4 M NaCl/ 0.1 mM PMSF. D1-D4 (709-886) was eluted from the column with PBS/2M Guanidine.HCl and then concentrated by stirred cell ultrafiltration, using a M_r 10,000 cut-off membrane (Amicon), to a 4.0 ml retentate. The D1-D4 (709-886) retentate was formulated into product by buffer

- exchange into PBS using two Sephadex G25 columns (PD10, Pharmacia). 1.5 mg >90% pure, determined by RP-HPLC and SDS PAGE, D1-D4 (709-886) product was obtained; material was confirmed as D1-D4 (709-886) by N-terminal sequencing and by Western blotting (probed with biotinylated fibronectin). The
- 5 molecular weight of the isolated, purified polypeptide, determined by electrospray mass spectrophotometry was 19,970. The theoretical molecular weight is 19,969. Molecular weight analysis according to SDS PAGE indicated that the D1-D4 (709-886) polypeptide had a mobility corresponding to a protein of approximately 35 000 (non-reduced) or approximately 40 000 (reduced); the protein markers used for this
- 10 were the Low Molecular Weight Kit (Pharmacia).
- (ii) Alternative purification method - D1-D4 (709-886) supernatant (from a 5L culture), prepared essentially as described in Example 1c, was diluted 1:1 in 0.1M NaH_2PO_4 pH 7.6 (final pH adjusted to 7.6) and applied to a Q Sepharose (Pharmacia) column (i.d., 7.8cm; h, 5cm) equilibrated in 0.1M NaH_2PO_4 pH 7.6.
- 15 D1-D4 (709-886) adsorbed to the column and was eluted using 0.1M NaH_2PO_4 / 0.5 M NaCl pH 7.6. It was then concentrated by stirred cell ultrafiltration, using a M_r 10,000 cut-off membrane (Amicon), to a 30ml retentate. At this stage D1-D4 (709-886) was approx. 50% pure. The D1-D4 (709-886) retentate was buffer exchanged into 50mM formic acid using a Sephadex G25 (Pharmacia) column
- 20 (i.d., 2.6cm; h, 21cm) to yield a 40ml product. The D1-D4 (709-886) product was further purified by four repeat runs on reverse-phase HPLC. Thus, 10ml D1-D4 (709-886) was applied to an Aquapore C4 column (Applied Biosystems) (i.d., 1cm; h, 10cm) equilibrated in 0.1% trifluoroacetic acid (TFA). D1-D4 (709-886) was eluted from the column using a 0 to 100% linear gradient of 0.085% TFA / 70% acetonitrile, over 4-5 column volumes. The appropriate D1-D4 (709-886)-
- 25 containing fractions from the four repeat runs were pooled and were concentrated by ultrafiltration (as above) to a 30ml retentate. The D1-D4 (709-886) retentate was formulated into final product by buffer exchange into 50mM formic acid (as above) followed by lyophilisation.
- 30 50mg \geq 95% pure, determined by analytical reverse-phase HPLC and SDS PAGE, D1-D4 (709-886) product was obtained; material was confirmed as D1-D4 (709-886) by N-terminal sequencing and by Western blotting (probed with biotinylated fibronectin). The solubility of the D1-D4 (709-886) lyophilised product was 35-40mg/ml when reconstituted in H_2O or 50mM formic acid.
- 35 (iii) Third purification method - this was developed to replace the C4 reverse-phase step. D1-D4 (709-886) (48ml) that had been eluted from a Q Sepharose column as described in (ii) was mixed with 4M $(\text{NH}_4)_2\text{SO}_4$ (16ml) and applied to a Toyopearl Butyl column (TosoHaas) (i.d., 1.6cm; h, 15cm) equilibrated in 1.0M

(NH₄)₂SO₄ / 0.1M NaH₂PO₄ pH7.0 (Buffer A). The column was then washed with approx. 3 bed volumes of Buffer A. The D1-D4 (709-886), which adsorbed to the matrix, was eluted from the column using a 30% to 100% linear gradient of 0.1M NaH₂PO₄ pH7.0 in Buffer A over 3 column volumes. The appropriate D1-D4 (709-886)-containing fractions were identified by SDS PAGE, were pooled and were then concentrated by ultrafiltration, using a M_r10,000 cut-off membrane (Amicon), to a 20ml retentate. The D1-D4 (709-886) retentate was formulated into final product by buffer exchange into 50mM formic acid using a Sephadex G25 column (Pharmacia) (i.d., 2.6cm; h, 21cm) followed by lyophilisation.

50mg ≥98% pure, determined by reverse-phase HPLC and SDS PAGE, D1-D4 (709-886) product was obtained.

Example 2 - Bioreactor fermentation of D1-D4 (709-886) polypeptide

Single colonies of E.coli BL21 (DE3):pBROC520 were recovered from LB agar medium containing 50µg/ml ampicillin and used to inoculate 2x100 ml of seed medium (NCYZM) containing ampicillin at 75µg/ml. The primary and secondary seed stage fermentations were carried out in 500ml shake flasks batched with 100ml aliquots of NCYZM medium. The primary and secondary seed fermentation conditions were as follows: 37°C, 230 rpm on an orbital shaker with a 50mm throw. The primary seed incubation time was 9 hours. The primary seed culture was used to inoculate (0.1% v/v) 6x100 ml aliquots of secondary seed stage medium (NCYZM). The secondary seed was incubated for 14.5 hours.

Two 15 litre Biolafitte fermenters were each batched with 10 litres of NCYZM medium and 0.01% (v/v) Dow Corning DC1510 antifoam. The vessels plus media were sterilised using steam to 121°C for 45 minutes. Ampicillin sterilised by microfiltration (0.2µm) was added aseptically to the vessel media to a final concentration of 150µg/ml. The fermenters were inoculated at a level of 2.5% (v/v) from pooled secondary seed culture. The final stage incubation conditions were 37°C, agitator 300 rpm, airflow 5 l/min (0.5vvm). The final stage fermentations were sampled aseptically pre-inoculation, at 0 hours and thence approximately hourly. The samples were monitored for increases in optical density (550nm). When the OD 550 was ≥ 1.0, IPTG was added to give a final concentration of 1mM. The fermentations were incubated for approximately a further 3 hours.

The cells were recovered by batch centrifugation using 7000g for 35 minute, or continuous centrifugation at 15,000g. The total cell yield was 73.5 grammes. Cells were washed once with a total of 1.0 litre of Oxoid phosphate buffered saline (Dulbecco 'A') pH 7.2. The centrifuged washed cells were then

held frozen at -20°C to await further processing.

Composition of NCYZM medium:

	Difco Bacto Tryptone:	10 g/l
5	Fisons AR NaCl:	5 g/l
	Difco Bacto Yeast Extract	5 g/l
	Difco Bacto Casamino Acids	1 g/l
	Fisons AR MgSO ₄ .7H ₂ O	2 g/l
	Deionised water	950ml
10	pH adjusted to 7.0 with 5M NaOH, then final volume to 1000ml.	

Example 3 - Expression, isolation and purification of D1-D4 (709-838(P838T)) polypeptide expressed from pBROC 533 in *E.coli* BL21(DE3)

15 a) **Expression** - This was carried out using the methods described in Example 1(a), except that plasmid pBROC 533 from Preparation 4 was substituted for pBROC520.

b) **Detection of expressed product** - These were carried out using the methods described in Example 1(b). D1-D4 (709-838(P838T)) was identified as a
20 new band in the pBROC 533 lane.

c) **Isolation** - This was carried out essentially as described in Example 1(c), except that cell pellets of *E.coli* BL21 (DE3) (pBROC 533) were used, the starting volume of culture was 1.2 litres and the cell pellet was resuspended in 32ml buffer. The final supernatant volume was 40 ml.

25 d) **Purification** - D1-D4 (709-838(P838T)) supernatant, prepared as described above, was diluted 1:1 with 0.1M NaH₂PO₄ pH7.6, the pH adjusted to 7.6 using HCl and applied to a Q Sepharose (Pharmacia) column (i.d., 4.1cm; h, 4.7cm) equilibrated in 0.1M NaH₂PO₄ pH7.6. The polypeptide adsorbed to the column and was eluted using 0.1M NaH₂PO₄ / 0.5M NaCl pH7.6. Polypeptide solution
30 (50ml) that had eluted from the Q Sepharose was mixed with 4M (NH₄)₂SO₄ (16ml) and applied to a Toyopearl Butyl column (TosoHaas) (i.d., 1.6cm; h, 15cm) equilibrated in 1.0M (NH₄)₂SO₄ / 0.1M NaH₂PO₄ pH7.0 (Buffer A). The column was then washed with approx. 3 bed volumes of Buffer A. The polypeptide, which adsorbed to the matrix, was eluted from the column using a 30% to 100% linear
35 gradient of 0.1M NaH₂PO₄ pH7.0 in Buffer A over 3 column volumes. The appropriate polypeptide-containing fractions were identified by SDS PAGE, were pooled and were then concentrated by ultrafiltration, using a M_r10,000 cut-off membrane (Amicon), to a 20ml retentate. The retentate was formulated into final

product by buffer exchange into 50mM formic acid using a Sephadex G25 column (Pharmacia) (i.d., 2.6cm; h, 21cm) followed by lyophilisation.

An aliquot of the lyophilisate was solubilised; the material showed a single major band of approximately $M_r=22000$ on SDS PAGE under non-reducing

5 conditions.

Example 4 - mouthwash formulation

Ethanol	12.00%
Flavour	0.12
Cremophor RH60	0.40
Sodium Fluoride	0.05
Soluble saccharin	0.05
Blue dye	0.075
Chlorophyllin	0.01
Polypeptide	0.3
Deionised water	qs

10 Example 5 - mouthwash formulation

Glycerin	5.00%
Flavour	0.12
Pluronic F-108	0.60
Cremophor RH60	0.40
Sodium Fluoride	0.05
Soluble saccharin	0.05
Blue dye	0.075
Chlorophyllin	0.01
Polypeptide	0.3
Deionised water	qs

Example 6 - Dentifrice

Glycerin	22.00
Hydroxypropyl methylcellulose	3.40
Titanium dioxide	1.00
Sodium saccharin	0.30
Poloxamer (Pluronic F108)	2.00
Flavour	1.00
Silica (RP93, Rhone-Poulenc)	16.00
Deionised water	qs

5 The dentifrice base may be used to provide a dentifrice comprising a D1D4 polypeptide 0.2, 0.5, 1, 1.5 or 2%.

Example 7 - Dentifrice

Sorbitol soln (70%)	29.00%
Calcium carbonate	35.00
Amorphous silica	2.00
Carrageenan	0.70
Carboxy methyl cellulose	0.60
Sodium lauryl sulphate	1.70
Sodium monofluorophosphate	0.80
Sodium hydroxide	0.102
Flavour	1.0
Sodium saccharin	0.38
Deionised water	qs

10

The dentifrice base may be used to provide a dentifrice comprising a D1D4 polypeptide 0.2, 0.5, 1, 1.5 or 2%.

Table 1 - A Comparison of the DNA Encoding the Fibronectin Binding Domains of *S. aureus* Fibronectin Binding Protein type A and of the *S. aureus* J2385 DNA sequence.

5	1	GGCCAAAATAGCGGTAACCAAGTCATTTCGAGGAAGACACAGAAGAAGATAA	50	J2385
	2350	GGCCAAAATAGCGGTAACCAAGTCATTTCGAGGAAGACACAGAAGAAGACAA	2399	Type A
	51	ACCTAAATATGAACAAGGTGGCAATATCGTAGATATCGATTTGATAGTG	100	J2385
10				
	2400	ACCTAAATATGAACAAGGTGGCAATATCGTAGATATCGATTTGATAGTG	2449	Type A
	101	TACCTCAAATTCATGGTCAAAATAAAGGTGATCAGTCATTCGAAGAAGAT	150	J2385
15	2450	TACCTCAAATTCATGGTCAAAATAAAGGTAATCAGTCATTCGAGGAAGAT	2499	Type A
	151	ACAGAGAAAGACAAGCCTAAATATGAACATGGTGGTAATATCATTGATAT	200	J2385
	2500	ACAGAGAAAGACAAGCCTAAATATGAACATGGTGGTAATATCATTGATAT	2549	Type A
20				
	201	CGACTTCGACAGCGTGCCACATATTCATGGATTCAATAAGCACACTGAAA	250	J2385
	2550	CGACTTCGACAGCGTGCCACATATTCATGGATTCAATAAGCACACTGAAA	2599	Type A
25	251	TTATTGAAGAAGATACAAACAAAGATAAACCAATTATCAATTCGGTGGA	300	J2385
	2600	TTATTGAAGAAGATACAAACAAAGATAAACCAAGTTATCAATTCGGTGGA	2649	Type A
	301	CACAATAGTGTGACTTTGAAGAAGATACACTCCACAAGTAAGTGGTCA	350	J2385
30				
	2650	CACAATAGTGTGACTTTGAAGAAGATACACTCCACAAGTAAGTGGTCA	2699	Type A
	351	TAATGAAGGTCAACAAACGATTGAAGAAGATACAAC-----GC	388	J2385
		*		
35	2700	AAATGAAGGTCAACAAACGATTGAAGAAGATACAACACCTCCAATCGTGC	2749	Type A
	389	CGCCAACACCACCAACACCAGAAGTACCAAGTGAGCCGGAACACCAACA	438	J2385
		+		
	2750	CACCAACGCCACCGACACCAGAAGTACCAAGTGAGCCGGAACACCAACG	2799	Type A
40				
	439	CCACCGACACCAGAAGTACCAAGTGAGCCGGAACACCAACACCGCCAAC	488	J2385
	2800	CCACCAACACCAGAAGTACCAAGTGAGCCGGAACACCAACACCGCGAC	2849	Type A
45	489	ACCAGAGGTACCAAGTGAGCCGGAACACCAACACC	524	J2385
	2850	ACCAGAAGTGCCGAGTGAGCCAGAACTCCAACACC	2885	Type A

+ Silent Mutation

* Change of Amino Acid

50

Table 2 - A comparison of the derived amino acid sequences of the Fibronectin Binding Regions of the Fibronectin Binding Proteins of *S aureus* (as published) and *S aureus* J2385.

- 5 The *S aureus* sequence 'a' comprises amino acid residues 709-886 (as in Signas *et al. op. cit.*)

		1 --> D1			--> D2	50
	J2385	GQNSGNQSFE	EDTEEDKPKY	EQGGNIVDID	FDSVPQIHGQ	NKGDQSFEED
10	Stapha	GQNSGNQSFE	EDTEEDKPKY	EQGGNIVDID	FDSVPQIHGQ	NKGNQSFEED
	Staphb	GQNSGNQSFE	EDTEEDKPKY	EQGGNIVDID	FDSVPQIHGQ	NNGNQSFEED
		51		--> D3		100
	J2385	TEKDKPKYEH	GGNIIDIDFD	SVPHINGFNK	HTEIIEEDTN	KDKPNYQFGG
15	Stapha	TEKDKPKYEH	GGNIIDIDFD	SVPHINGFNK	HTEIIEEDTN	KDKPSYQFGG
	Staphb	TEKDKPKYEQ	GGNIIDIDFD	SVPHINGFNK	HTEIIEEDTN	KDKPNYQFGG
		101	--> D4		--> WR1	146
	J2385	HNSVDFEEDT	LPQVSGHNEG	QQTIEEDTT-	---PPTPPTP	EVPSEPETPT
20	Stapha	HNSVDFEEDT	LPKVSGQNEG	QQTIEEDTTP	PIVPPTPPTP	EVPSEPETPT
	Staphb	HNSVDFEEDT	LPQVSGHNEG	QQTIEEDTTP	PIVPPTPPTP	EVPSEPETPT
				174		
	J2385	PPTPEVPSEP	ETPTPPTPEV	PSEPETPT		
25	Stapha	PPTPEVPSEP	ETPTPPTPEV	PSEPETPT		
	Staphb	PPTPEVPSEP	ETPTPPTPEV	PTEP----		

Figure - Fig.1. is a diagrammatic representation of plasmid pBROC413. *Bla* indicates the ampicillin resistance gene. $\phi 10$ the T7 RNA polymerase promoter and rbs the ribosome binding site. Arrows for $\phi 10$ and *bla* give the direction of transcription. The polylinker site has been indicated. The plasmid is not drawn to scale and the size is approximate.

5

Sequence Listing - Key

SEQ ID NO:1 FIB 1

GGGAATTCAT ATGGGCCAAA ATAGCGGTAA CCAGTC

5

SEQ ID NO:2 FIB 2

GCGGATCCTT ACGTTGGTGG CACGATTGGA GGTG

SEQ ID NO:3 oligonucleotide (A)

10 CGGAATTCGT CAACAAACGA TTGAAGAAGA TACAACGACG TAAGATCTGG
ATCCGCATGC GAATTCCG

SEQ ID NO:4 oligonucleotide (B)

CGGAATTCGT CAACAAACGA TTGAAGAAGA TACAACGCCG TAAGATCTGG
15 ATCCGCATGC GAATTCCG

SEQ ID NO:5

GGCCAAAATA GCGGTAACCA GTCATTGAG GAAGACACAG AAGAAGATAA
ACCTAAATAT GAACAAGGTG GCAATATCGT AGATATCGAT TTCGATAGTG
20 TACCTCAAAT TCATGGTCAA AATAAAGGTG ATCAGTCATT CGAAGAAGAT
ACAGAGAAAG ACAAGCCTAA ATATGAACAT GGTGGTAATA TCATTGATAT
CGACTTCGAC AGCGTGCCAC ATATTCATGG ATTCAATAAG CACACTGAAA
TTATTGAAGA AGATACAAAC AAAGATAAAC CAAATTATCA ATTCGGTGGA
CACAATAGTG TTGACTTTGA AGAAGATACA CTTCCACAAG TAAGTGGTCA
25 TAATGAAGGT CAACAAACGA TTGAAGAAGA TACAACGCCG CCAACACCAC
CAACACCAGA AGTACCAAGT GAGCCGGA CACCAACACC ACCGACACCA
GAAGTACCAA GTGAGCCGGA AACACCAACA CCGCCAACAC CAGAGGTACC
AAGTGAGCCG GAAACACCAA CACCTCCAAT CGTGCCACCA ACGTAA

30 SEQ ID NO:6 D1-D4 (709-886)

GQNSGNQSFE EDTEEDKPKY EQGGNIVDID FDSVPQIHGQ NKGDQSFEED
TEKDKPKYEH GGNIIDIDFD SVPHIHGFNK HTEIIEEDTN KDKPNYQFGG
HNSVDFEEDT LPQVSGHNEG QQTIEEDTTP PTPPTPEVPS EPETPTPTPT
EVPSEPETPT PPTPEVPSEP ETPTPIVPP T

35

SEQ ID NO:7 D1-D4 (709-838(P838T)) (Example 7)

GQNSGNQSFE EDTEEDKPKY EQGGNIVDID FDSVPQIHGQ NKGDQSFEED
TEKDKPKYEH GGNIIDIDFD SVPHIHGFNK HTEIIEEDTN KDKPNYQFGG

HNSVDFEEDT LPQVSGHNEG QQTIEEDTTT

SEQ ID NO:8 D1-D4 (709-838)

GQNSGNQSFE EDTEEDKPKY EQGGNIVDID FDSVPQIHGQ NKGDQSFEED
 5 TEKDKPKYEH GGNIIDIDFD SVPHINGFNK HTEIIIEEDTN KDKPNYQFGG
 HNSVDFEEDT LPQVSGHNEG QQTIEEDTTP

SEQ ID NO:9 J2385 DNA (Table 1)

GGCCAAAATA GCGGTAACCA GTCATTCGAG GAAGACACAG AAGAAGATAA
 10 ACCTAAATAT GAACAAGGTG GCAATATCGT AGATATCGAT TTCGATAGTG
 TACCTCAAAT TCATGGTCAA AATAAAGGTG ATCAGTCATT CGAAGAAGAT
 ACAGAGAAAG ACAAGCCTAA ATATGAACAT GGTGGTAATA TCATTGATAT
 CGACTTCGAC AGCGTGCCAC ATATTCATGG ATTCAATAAG CACTGAAA
 TTATTGAAGA AGATACAAAC AAAGATAAAC CAAATTATCA ATTCGGTGGA
 15 CACAATAGTG TTGACTTTGA AGAAGATACA CTTCCACAAG TAAGTGGTCA
 TAATGAAGGT CAACAAACGA TTGAAGAAGA TACAACGCCG CCAACACCAC
 CAACACCAGA AGTACCAAGT GAGCCGAAA CACCAACACC ACCGACACCA
 GAAGTACCAA GTGAGCCGGA AACACCAACA CCGCCAACAC CAGAGGTACC
 AAGTGAGCCG GAAACACCAA CACC

20

SEQ ID NO:10 J2385 (Table 2)

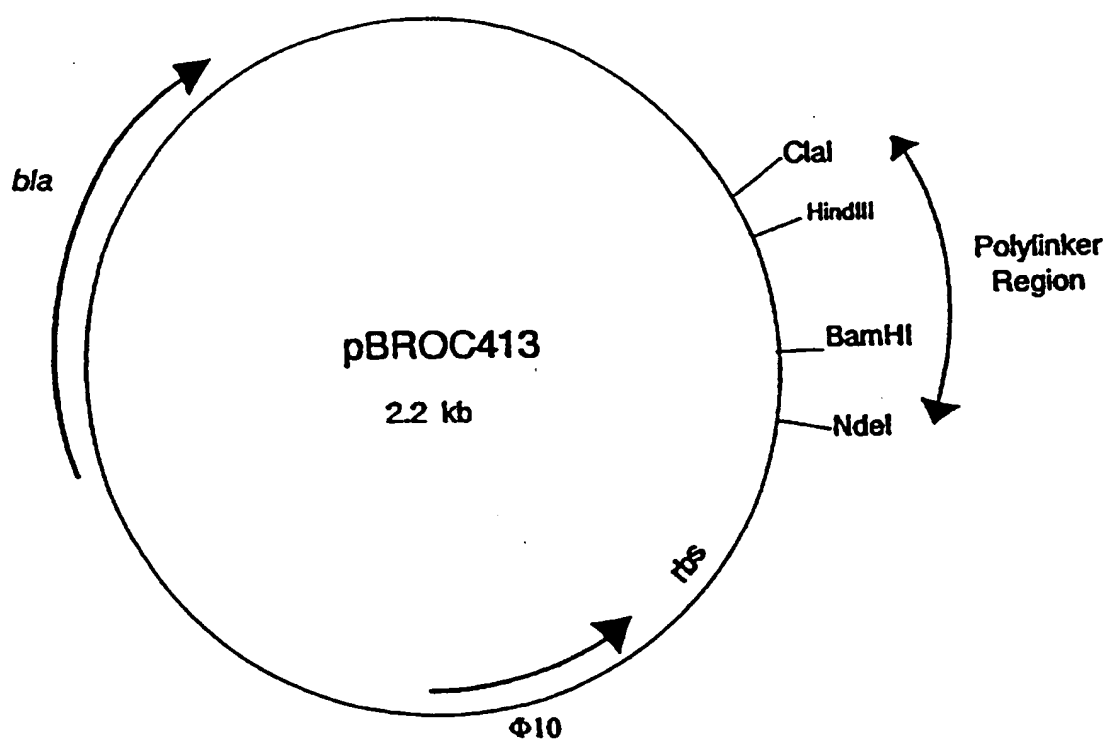
GQNSGNQSFE EDTEEDKPKY EQGGNIVDID FDSVPQIHGQ NKGDQSFEED
 TEKDKPKYEH GGNIIDIDFD SVPHINGFNK HTEIIIEEDTN KDKPNYQFGG
 HNSVDFEEDT LPQVSGHNEG QQTIEEDTTP PTPPTPEVPS EPETPTPTPT
 25 EVPSEPETPT PPTPEVPSEP ETPT

Claims

1. A method of preventing the adherence of oral pathogens to surfaces in the oral cavity, in particular tooth surfaces, which method comprises applying a
5 fibronectin binding protein or polypeptide to a patient in need thereof.
2. A method of preventing the adherence of oral pathogens to surfaces in the oral cavity, in particular tooth surfaces, which method comprises applying a
10 monoclonal antibody, or a fragment thereof, that binds to one or more epitopes of a matrix binding protein, such as fibronectin binding protein, to block the adhesion of oral pathogens to the matrix protein to a patient in need thereof.
3. The use of a fibronectin binding protein or polypeptide in the manufacture of an
15 oral hygiene composition for preventing the adherence of oral pathogens to surfaces in the oral cavity, in particular tooth surfaces.
4. The use of a monoclonal antibody, or fragment thereof, that binds to one or
20 more epitopes of a matrix binding protein, such as fibronectin binding protein in the manufacture of a oral hygiene composition for preventing the adherence of oral pathogens to surfaces in the oral cavity, in particular tooth surfaces.
5. A method or use according to claims 1 or 3 wherein the fibronectin binding protein is an isolated D1-D4 polypeptide.
- 25 6. An oral hygiene composition comprising an above mentioned fibronectin binding protein or polypeptide, in particular an isolated D1-D4 polypeptide or Mab or active fragment and an orally acceptable carrier.
- 30 7. An oral hygiene composition according to claim 5 comprising a further oral hygiene agent, such as an anticaries, antiplaque, anticalculus and/or antisensitivity agent.

1/1

Fig. 1



INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/EP 95/02825

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K38/16 A61K7/16 A61K39/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 163 623 (ALFA LAVAL AGRI INT) 4 December 1985 cited in the application see page 1, line 3 - page 3, line 15 ---	1-7
X	EP,A,0 294 349 (ALFA LAVAL AGRI INT) 7 December 1988 cited in the application see page 2, line 16 - page 3, line 1 see page 8, line 1 - page 9, line 13 ---	1-7
X	EP,A,0 397 633 (ALFA LAVAL AGRI INT) 14 November 1990 cited in the application see page 3, line 16 - line 46 see page 12, line 5 - line 30 ---	1-7
	--- -/--	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

18 October 1995

Date of mailing of the international search report

24. 11. 95

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

Intern. Appl. Application No

PCT/EP 95/02825

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 86, January 1989 pages 699-703, SIGNXS ET AL 'NUCLEOTIDE SEQUENCE OF THE GENE FOR A FIBRONECTIN-BINDING PROTEIN FROM STAPHYLOCOCCUS AUREUS:USE OF THIS PEPTIDE SEQUENCE IN THE SYNTHESIS OF BIOLOGICALLY ACTIVE PEPTIDES' cited in the application see the whole document ----	6
X	DATABASE MEDLINE FILE SERVER STN KARLSRUHE ABSTRACT NO.90338990, LINDAHL ET AL 'ADHESIVE PROTEINS OF HAEMAGGLUTINATING STAPHYLOCOCCUS AUREUS ISOLATED FROM BOVINE MASTITIS' see abstract & J GEN MICROBIOL, vol. 136, no. PT 5, May 1990 pages 935-939, ----	6
A	WO,A,92 06191 (PROTEIN ENGINEERING CORP) 16 April 1992 ----	
A	GB,A,2 139 635 (NAT RES DEV) 14 November 1984 cited in the application & WO,A,84 04546 (NAT RES DEV) ----	
P,X	WO,A,94 18327 (SMITHKLINE BEECHAM PLC ;BURNHAM MARTIN KARL RUSSEL (GB); CHOPRA IA) 18 August 1994 cited in the application see page 1, line 3 - page 3, line 21 see page 9, line 31 - page 11, line 3 -----	1-7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 95/ 02825

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1,2 and 5 partially are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Intern. Appl. No.

PCT/EP 95/02825

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		WO-A- 8505553	19-12-85
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		DE-T- 3853446	27-07-95
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